AD-A206 247

DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED

1b. RESTRICTIVE MARKINGS: NONE

2a. SECURITY CLASSIFICATION AUTHORITY:

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE: N/A

3. DISTRIBUTION/AVAILABILITY OF REPORT: APPROVED FOR PUBLIC RELEASE; UNLIMITED DISTRIBUTION

4. PERFORMING ORGANIZATION REPORT NUMBER(S): USA RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE, NATICK, MA 01760-5007

5. MONITORING ORGANIZATION REPORT NUMBER(S):

6a. NAME OF PERFORMING ORGANIZATION: USARIEM, NATICK, MA 01760-5007

6b. OFFICE SYMBOL (If applicable): SGRD-UE-CR

7a. NAME OF MONITORING ORGANIZATION: SAME AS 4

6c. ADDRESS (City, State, and ZIP Code): USA RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE, NATICK, MA 01760-5007

7b. ADDRESS (City, State, and ZIP Code):

8a. NAME OF FUNDING/SPONSORING ORGANIZATION:

8b. OFFICE SYMBOL (If applicable):

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER:

8c. ADDRESS (City, State, and ZIP Code):

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
	3M16110 2BS15	S15/CA	051

11. TITLE (Include Security Classification): (U) IN SITU AND IN VITRO COMPARISONS OF ENDOTHELIAL CELL G- AND F-ACTIN DISTRIBUTION

12. PERSONAL AUTHOR(S): DAVID A. DUBOSE, ROBERT J. CARPENTER, JR., JOSE A. GUZMAN AND ROSARIA HAUGLAND

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
MANUSCRIPT	FROM _____ TO _____	5FEB89	16

16. SUPPLEMENTARY NOTATION:

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	ENDOTHELIAL CELLS, CYTOSKELETON, F-ACTIN ACTIN PROBES

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Numerous studies have described the F-actin cytoskeleton, however, little information relevant to G-actin is available. Using in situ and in vitro conditions and rhodamine-conjugated probes specific for G- (deoxyribonuclease 1, 0.3 uM) or F-actin (phallaidin, 0.2 uM), the actin pools of bovine aortic endothelial cells were examined. Cells in situ displayed a diffuse G-actin distribution, while F-actin was concentrated in the ectoplasm and in fine stress fibers that traversed some cells. Cells of subconfluent or just confluent in vitro cultures demonstrated intense fluorescence, with many F-actin stress fibers. Post confluent cultures resembled the in situ condition, since peripheral F-actin was prominent, traversing actin stress fibers were greatly reduced and fluorescent intensity was diminished. Post confluency had little influence on G-actin, with only an enhancement in the intensity of G-actin punctate fluorescence. When post confluent cultures were treated with cytochalasin-D (15 min; 10^{-4} M), F-actin networks were disrupted and F-actin punctate and diffuse fluorescence increased. G-actin fluorescence was not altered by this disruption. Though its unstructured nature may account for the minor changes observed, the stability of the G-actin pool in the

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION: UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL: DAVID A. DUBOSE, Ph.D.

22b. TELEPHONE (Include Area Code): (508)651-4264

22c. OFFICE SYMBOL: SGRD-UE-CR

DD Form 1473, JUN 86 — Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE: UNCLASSIFIED

DTIC

DISPOSITION FORM

For use of this form, see AR 340-15; the proponent agency is TAGO.

REFERENCE OR OFFICE SYMBOL	SUBJECT
SGRD-UE	Request for Clearance of Technical Paper (USARIEM)

TO Commander, USARIEM FROM Director, Cold Research DATE 27 Jan 89 CMT 1

1. Reference USARIEM Memo 360-1, request clearance of attached [X] manuscript, [ ] abstract, [ ] presentation, [ ] technical report, [ ] review article. Report Documentation Page, DD Form 1473 (is)(is not) attached.

Title In Situ and In Vitro Comparisons of Endothelial Cell G- and F-actin Distribution

Author(s) David A. DuBose, Robert J. Carpenter, Jr., Jose A. Guzman and Rosaria Haugland

Intended for publication in Journal of Histochemistry and Cytochemistry

Intended for presentation before _____

_____ Location _____ Date _____

2. Budget Project No. 3M161102BS15 Cost Code 9470082101059

3. Attached contains no classified material. It meets accepted standards for scientific accuracy and propriety. It contains no potentially sensitive or controversial items.

MURRAY P. HAMLET, D.V.M.
Director
Cold Research Division

Encl

SGRD-UEZ ( )

THRU Chief, Admin Svc Br FROM Commander DATE 5 Feb 89 CMT 2

TO Ch Cold Res. Div

[X] Clearance is granted.

[ ] Clearance is not granted.

[ ] This document must be forwarded to USAMRDC for clearance.

DAVID D. SCHNAKENBERG
Colonel, MS
Commanding

Encl
nc

CLEARANCE NO. M24-89

NATICK FL 453
1 Jun 86 EDITION OF 1 DEC 84 IS OBSOLETE.

DA FORM 2496 AUG 86 PREVIOUS EDITIONS WILL BE USED

DD Form 1473 Continued - Block 19:

presence of notable F-actin modulations suggested that filamentous actin was the key constituent involved in these actin cytoskeletal alterations.

Running Heading:

G- and F-actin distribution in endothelial cells

In Situ and In Vitro Comparisons of Endothelial Cell G- and F-actin Distribution

David A. DuBose[1], Robert J. Carpenter, Jr., Jose A. Guzman and Rosaria Haugland[2]

U.S. Army Research Institute of Environmental Medicine, Natick, MA 01581

[1] Correspondence to:
D.A. DuBose, Ph.D.
Experimental Pathology Branch
Cold Research Division
USARIEM
Natick, MA 01760
(508) 651-4861

[2] Molecular Probes, Inc.
24750 Lawrence Road
Junction City, OR 97448

ABSTRACT

Numerous studies have described the F-actin cytoskeleton, however, little information relevant to G-actin is available. Using in situ and in vitro conditions and rhodamine-conjugated probes specific for G- (deoxyribonuclease I, 0.3 μM) or F-actin (phalloidin, 0.2 μM), the actin pools of bovine aortic endothelial cells were examined. Cells in situ displayed a diffuse G-actin distribution, while F-actin was concentrated in the ectoplasm and in fine stress fibers that traversed some cells. Cells of subconfluent or just confluent in vitro cultures demonstrated intense fluorescence, with many F-actin stress fibers. Post confluent cultures resembled the in situ condition, since peripheral F-actin was prominent, traversing actin stress fibers were greatly reduced and fluorescent intensity was diminished. Post confluency had little influence on G-actin, with only an enhancement in the intensity of G-actin punctate fluorescence. When post confluent cultures were treated with cytochalasin-D (15 min; 10^{-4} M), F-actin networks were disrupted and F-actin punctate and diffuse fluorescence increased. G-actin fluorescence was not altered by this disruption. Though its unstructured nature may account for the minor changes observed, the stability of the G-actin pool in the presence of notable F-actin modulations suggested that filamentous actin was the key constituent involved in these actin cytoskeletal alterations.

INTRODUCTION

There are several pools of actin within nonmuscle cells (16). Polymerized actin comprises the filamentous pool (F-actin), while monomeric actin forms the globular pool (G-actin). In addition, F-actin interacts with other contractile proteins to from complexes known as stress fibers. A number of qualitative studies have defined the distribution of F-actin and stress fibers in vascular endothelial cells under various conditions (7,8,15,21-24,26). However, there is little qualitative information concerning F-actin and stress fiber arrangement relative to the distribution of G-actin. This information is necessary to verify quantitative findings and to better understand the degree of interaction among the various pools of actin. Such an understanding is important, since actin cytoskeletal changes are reported to influence such diverse endothelial cell activities as motility (4), permeability (3,21) and metabolism (5,6).

A widely used approach for the visualization of the F-actin pool employes the capacity of the fluorescently labeled mushroom toxin, phalloidin, to bind specifically with polymerized actin (25). In this report, a similar approach has been employed to observe G-actin. Deoxyribonuclease I (DNase I) interacts specifically with this protein (13,17) and when conjugated with rhodamine, DNase I can serve as a fluorescent probe for G-actin. The current study describes the distribution of the actin pools in bovine aortic endothelial cells (BAEC) in situ and in vitro.

MATERIALS and METHODS

The in situ preparations were obtained as previously described (19). Bovine aortae were cleaned of adventitia, rinsed in Hanks balanced salt solution (HBSS; GIBCO, Grand Island, NY), treated with 100% methanol (-20° C; 4 min) and exposed to 100% acetone (-20° C; 2 min). After fixation, the vessels were washed in HBSS, cut into 1/2 cm^2

sections and dried in a stream of freon. Next, a section was placed, endothelium side down, on a cover slip that was positioned on an aluminum plate cooled (-60° C) in a dry ice/ethanol bath. When ice crystals formed in a water drop placed near the segment, the segment was pried free to leave the endothelium attached to the cover slip.

The in vitro culture of BAEC employed modifications of procedures previously described (14). The aorta was rinsed in HBSS and a lateral cut was made to expose the tunica intima. A sterile scalpel blade was used to lightly scrape the endothelial cells from the vessel. Scrapings were deposited into collagenase (1mg/ml; Cooper Biomedical, Malvern, PA) and incubated at 37° C for 10 min. Four mls of supplemented [10% fetal calf serum, penicillin-streptomyocin (100units/ml-100μg/ml), and glutamine (0.29mg/ml); GIBCO, Grand Island, NY] Dulbecco's modified Eagle's Medium (DMEM; GIBCO, Grand Island, NY) were then added. This was centrifuged at 3000g (5 min). The fluid was aspirated, the pellet suspended in supplemented DMEM (5 ml) and the solution pipetted into a tissue culture flask (Falcon 3013; Becton Dickinson, Lincoln Park, NJ). Cells were maintained on supplemented DMEM until they reached confluency. The culture was then rinsed with HBSS and the cells removed with trypsin (0.3 ml; 0.5 mg/ml; GIBCO, Grand Island, NY). Cell number was determine and serial dilutions in supplemented DMEM made to obtain a solution with 10 cells/ml. A 0.5 ml volume was placed in each well of a 24 well tissue culture plate (Falcon 3047; Becton Dickinson, Lincoln Park, NJ). In addition to supplemented DMEM, these cells were maintained with endothelial cell growth supplement (30 μg/ml; Collaborative Research, Bedford, MA) until small patches of growth were visible by microscopic examination. After this point, cells were maintained with only DMEM. Cultures were observed for 2 weeks after obtaining confluency to ensure that they were free of smooth muscle contaminants, after which they were removed with trypsin, seeded into tissue culture flasks and grown to confluency. Cells were then harvested and stored in an environment of liquid nitrogen until needed. Cultures demonstrated a "cobblestone" pattern typical for endothelial cells and were

positive for the presence of factor VIII antigen by immunofluorescent techniques. From this stock, seedings were made on glass cover slips at a density of 1.5×10^4 cells/cm^2. After 3,6,10,20 and 32 days of culture, the cells were fixed in 3.7% formalin. In other studies, post confluent cultures (day 9) were treated with cytochalasin-D (15 min; 10^{-4} M) to alter F-actin arrangement and then fixed.

Rhodamine conjugated phalloidin (R-P) and DNase I (R-DNase I) were obtained from Molecular Probes (Junction City, OR) and used to delineate the F- and G-actin pools, respectively. To permit probe entry, the endothelial cell membranes were permeabilized with 80% acetone (10 min). Cells were then treated with R-P (0.2 μM) or R-DNase I (0.3μM) for 10 min, after which the culture was washed (HBSS) 5 times. A Zeiss microscope equipped with a 40X water immersion objective and filters appropriate for rhodamine excitation were used to observe the cell fluorescence. Photographs of the fluorescence were taken using Tri-X Pan film (Kodak; 400 ASA). Automatic exposure times were employed for the photographs of BAEC in situ. When in vitro post confluency state was studied, film exposure time was set to that noted for cultures at their first day of confluency (day 6). Exposure time for print development also used a setting noted for day 6 cultures. In this manner, changes in fluorescent intensity could be depicted. In the study of cytochalasin-D effects, the film and print exposure times were set relative to that noted for cultures prior to cytochalasin-D treatment.

RESULTS

A comparison of the in situ G- and F-actin distributions of BAEC is illustrated in figure 1. G-actin appeared to be distributed in a diffuse manner, with enhanced fluorescence near the nucleus, such that this organelle was highlighted in the photographs (Fig. 1a). Fluorescent intensity associated with in vitro G-actin (Figs. 2a-e) was greater than that noted for the in situ condition. However, the BAEC in situ and in vitro G-

actin distributions were similar, since both were diffuse. The fluorescence associated with in vitro G-actin was enhanced in the region of the cell nucleus and diminished in the ectoplasm, which also demonstrated the similarities of the G-actin distributions in vitro and in situ. Other than the appearance of areas of bright punctate fluorescence first noted on day 20 (Fig. 2d) that increased by day 32 (Fig. 2e), changes in BAEC confluency state had little effect on the distribution of in vitro G-actin.

A low level of fluorescence was associated with in situ F-actin (Fig. 1b). F-actin was concentrated in the cell ectoplasm and outlined the cell periphery. Some cells also demonstrated the presence of fine stress fibers composed of F-actin that traversed the cell. In contrast, the F-actin fluorescent intensity was greatly increased for cultured cells (Figs. 2f,g). Many F-actin stress fibers could be seen traversing the cells and in the ectoplasm. Unlike in vitro G-actin, the in vitro F-actin distribution was dramatically altered by the state of confluency. Subconfluent BAEC cultures (day 3; Fig. 2f) had cells with many stress fibers, while others had a more diffuse distribution with few stress fibers. Cultures at that their first day of confluency (day 6; Fig. 2g) demonstrated intense fluorescence and numerous F-actin stress fibers consumed most cells. Four days post confluency (day 10; Fig. 2h), there was an increased presence of areas within the monolayer in which fluorescent intensity was diminished and stress fiber density decreased. Fourteen days post confluency (day 20; Fig. 2i), these areas were more prominent. Other than differences in their shape and size, BAEC now resembled those seen in situ, since F-actin was generally noted in the ectoplasm and few F-actin stress fibers could be demonstrated. Observations made at 32 days in culture (Fig. 2j) did not indicate any further changes in the fluorescent intensity or distribution of F-actin.

G-actin was not affected by exposure to cytochalasin-D, since it remained diffusely distributed and of similar fluorescent intensity (Figs. 3a,b). This was in contrast to the effects of such exposure on the F-actin pool (Figs. 3c,d). F-actin stress fiber networks

were disrupted and there was an increase in diffuse and punctate fluorescence within the cells (Fig. 3d).

DISCUSSION

There is a paucity of qualitative information concerning F-actin distribution relative to that of G-actin. Actin quantitative studies have been reported using the DNase I inhibition test (1,2,9-11). However, these studies rely on the effectiveness of buffer systems to ensure that actin polymerization state remains constant, when the amount of actin comprising the F-actin pool is determined. The failure to maintain the actin polymerization state may be a source of error and account for some inconsistencies in the quantitative findings (9). Qualitative studies may aid not only our understanding of the interaction between the various pools of actin, but help to clarify quantitative findings. This report illustrates the use of fluorescently labelled DNase I to visualize the G-actin pool relative to that of F-actin.

As might be expected for an unstructured, monomeric form of a protein, G-actin distribution was diffuse in nature both in situ and in vitro. As noted previously for in vitro F-actin (20), the level of fluorescence associated with in vitro G-actin was greater than that noted for the in situ condition. This indicated that cultured cells may have more G-actin. Though there was the appearance of an increased fluorescence near the nucleus, this may not represent an area of increased G-actin concentration, since the region adjacent to the nucleus is of greatest cytoplasmic thickness and increased fluorescence would be expected when viewing through such an area. Thus, G-actin was noted throughout the cell and was perhaps of similar concentration in the various cellular regions.

In contrast, the in situ arrangement of F-actin, was concentrated in the region of the ectoplasm and in stress fibers. This confirmed previous findings on the location of F-actin in endothelial cells studied directly from vessels (6,7,12,19,20,23,24,26). When cultured under in vitro conditions, the F-actin arrangement is quite distinct from endothelial cells in situ (20). As verified by this study, there was an increase in actin stress fibers, which were circumferential and traversed the cell (Figs. 2f,g). However, with prolonged culture ($\geq$10 days; Figs. 2h-j), the in vitro F-actin arrangement more closely approximated the in situ condition. This finding was relative to the shorter culture periods (day 6; Fig. 2g), since photographic exposure times for the long term cultures were the same as those used for the short term cultures. If automatic exposure times had been employed, the longer term cultures would appear to have had a greater fluorescent intensity and the F-actin arrangement would not have been accurately depicted. Though this change (day 10; Fig. 2h) was not uniformly noted, areas of reduced F-actin and stress fiber density did become more pronounced with time, such that by day twenty (Fig. 2i) this F-actin distribution predominated and no further changes were noted (day 32; Fig. 2j). Quantitative studies indicate that F-actin levels can be reduced in some cell lines after obtaining a state of confluence (11), which these qualitative findings supported. Such findings suggested that endothelial cells in culture might best be studied when maintained for a sufficient period to allow for an F-actin distribution more in keeping with that seen in the natural state.

In spite of notable changes in F-actin arrangement, culture confluency state had little observable impact on G-actin distribution. G-actin remained diffusely dispersed with only the appearance of enhanced punctate fluorescence noted after twenty days of culture (Figs. 2d,e). Since its appearance (day 20) did not coincide with the first sign of a reduction in F-actin stress fiber density (day 10; Fig. 2h) and became more pronounced (day 32; Fig. 2e) in the absence of any further alterations in the F-actin pool (day 32; Fig. 2j), the enhancement of punctate fluorescence associated with G-actin seemed

independent of the modulations in F-actin distribution. Thus, while F-actin arrangement was dramatically altered by confluency state, it did not appear to directly affect G-actin. When cytochalasin-D treatment was used to alter F-actin arrangement, the effect on the G-actin pool was also not recognizable (Fig. 3). This corroborates earlier quantitative findings that cytochalasin-D treatment of human tumor cell lines does not result in net depolymerization of actin filaments (18). Thus, the appearance of increased diffuse and punctate fluorescence associated with F-actin (Fig. 3d) indicated that the F-actin of the disrupted stress fibers may have coalesced into these areas and did not depolymerize to increase the G-actin pool.

In conclusion, alterations in the distribution of F-actin by confluency state or cytochalasin-D treatment did not appear to significantly influence the G-actin pool. Though changes in G-actin may be too subtle to observe due to the unstructured nature of this monomeric protein, these results suggested that F-actin alterations occurred independent of substantial effects on the G-actin pool. Moreover, since shifts in the arrangement of F-actin and actin stress fibers were more noteworthy than qualitative changes in G-actin, filamentous actin might be the major player in cellular functions influenced by actin. This perhaps has special importance to the comprehension of the modulations in endothelial cell prostacyclin production associated with changes in actin arrangement (5,6), since it would suggest that F-actin and stress fibers have a greater role in this metabolic regulation than does G-actin. The use of G- and F-actin probes might facilitate the investigation of the relative contributions made by the actin pools in such actin-mediated phenomena.

REFERENCES

1. Blikstad I, Carlsson L: On the dynamics of the microfilament system in HeLa cells. J Cell Biol 93:122, 1982

2. Bilkstad I, Markely F, Carlsson L, Persson T, Linberg U: Selective assay of monomeric and filamentous actin in cell extracts, using inhibition of deoxyribonuclease I. Cell 15:935, 1978

3. Bottaro D, Shepro D, Peterson S, Hechtman HB: Serotonin, norepinephrine, and histamine mediation of endothelial cell barrier function in vitro. J Cell Physiol 128:189, 1986

4. Bottaro D, Shepro D, Peterson S, Hechtman HB: Serotonin, norepinephrine, and histamine mediation of endothelial cell and vascular smooth muscle cell movement. Am J Physiol 248 (Cell Physiol 17):C252, 1985

5. DuBose DA, Shepro D, Hechtman HB: Correlation among endothelial cell shape, F-actin arrangement, and prostacyclin synthesis. Life Sci 40:447, 1987

6. DuBose DA, Shepro D, Hechtman HB: Modulation of phospholipase A_2 lytic activity by actin and myosin. Inflammation 13:15, 1989

7. Gabbiani G, Badonnel M-C, Rona G.: Cytoplasmic contractile apparatus in aortic endothelial cells of hypertensive rats. Lab Invest 32:227, 1975

8. Gabbiani G, Gabbiani F, Lombardi, D., Schwartz SM: Organization of actin cytoskeleton in normal and regenerating arterial endothelial cells. Proc Natl Acad Sci USA 80:2361, 1983

9. Heacock CS, Bamberg JR: The quantitation of G- and F-actin in cultured cells. Anal Biochem 135:22, 1983

10. Heacock CS, Bamberg JR: Levels of filamentous and globular actin in chinese hamster ovary cells throughout the cell cycle. Exp Cell Res 147:240, 1983

11. Heacock CS, Eidsvoog KE, Bamberg JR: The influence of contact-inhibited growth and agent which alter cell morphology on the levels of G- and F-actin in cultured cells. Exp Cell Res 153:402, 1984

12. Herman IM, Pollard TD, AJ Wong: Contractile proteins in endothelial cells. Ann NY Acad Sci 401:50, 1982

13. Hitchcock SE: Actin deoxyribonuclease I interaction. Depolymerization and nucleotide exchange. J Biol Chem 255:5668, 1980

14. Jaffe EA, Nachman RL, Becker GC, Minick CR: Culture of human endothelial cells derived from umbilical veins: Identification by morphologic and immunologic criteria. J Clin Invest 32:2745, 1973

15. Kalnins VI, Subrahmanyan L, Gotlieb AI: The reorganization of cytoskeletal fibre systems in spreading porcine endothelial cells in culture. Eur J Cell Biol 24:36, 1981

16. Korn ED: Actin polymerization and its regulation by proteins from nonmuscle cells. Physiol Rev:62:672, 1982

17. Mannherz HG, Goody RS, Konrad M, Nowak E: The interaction of bovine pancreatic deoxyribonuclease I and skeletal muscle actin. Eur J Biochem 104:367, 1980

18. Morris A, Tannenbaum J: Cytochalasin D does not produce net depolymerization of actin filaments in HEp-2 cells. Nature 287:637, 1980

19. Rogers KM, Kalnins V: A method for examining the endothelial cytoskeleton in situ using immunofluorescence. J Histochem Cytochem 31:1317, 1983

20. Rogers KM, Kalnins V: Comparison of the cytoskeleton in aortic endothelial cells in situ and in vitro. Lab Invest 49:650, 1983

21. Welles SL, Shepro D, Hechtman HB: Vasoactive amines modulate actin cables (stress fibers) and surface area in cultured bovine endothelium. J Cell Physiol 123:337, 1985

22. Welles SL, Shepro D, Hechtman HB: Eicosanoid modulation of stress fibers in cultured bovine aortic endothelial cells. Inflammation 9:439, 1985

23. White GE, Fujiwara K: Expression and intracellular distribution of stress fibers in aortic endothelium. J Cell Biol 103:63, 1986

24. White GE, Gimbrone MA, Fujiwara K: Factors influencing the expression of stress fibers in vascular endothelial cells in situ. J Cell Biol 97:416, 1983

25. Weiland T, Faulstich H: Amatoxins, phallotoxins, phallolysin and antaminide: The biologically active components of poisonous amanita mushrooms. CRC Crit Rev Biochem 5:185, 1978

26. Wong AJ, Pollard TD, IM Herman: Actin filaments stress fibers in vascular endothelial cells in vivo. Science 219:867, 1983

Fig. 1. BAEC fixed in situ and exposed to R-DNase I (a) or R-P (b) to demonstrate the distribution of G- or F-actin, respectively. G-actin (a) was diffusely distributed, but appeared concentrated in the area of the cell nucleus (n). F-actin was concentrated in the cell ectoplasm and highlighted the cell periphery. Some cells also showed thin, F-actin stress fibers (arrows) that traversed the cell. Bar=10μ

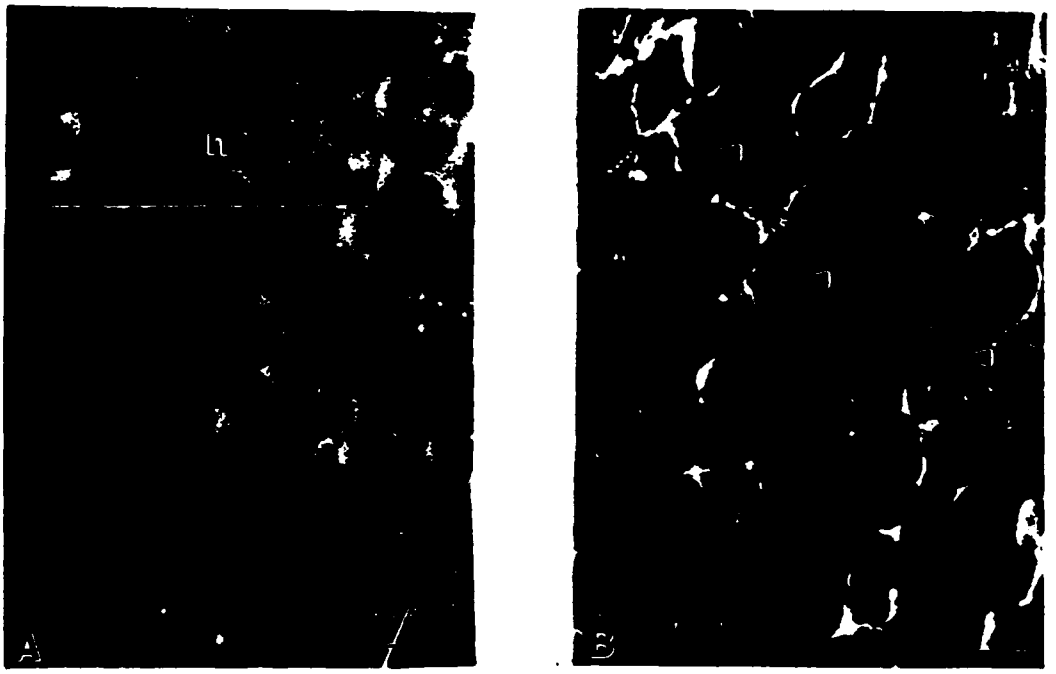
A
B

Fig. 2. BAEC examined at various points of confluency under in vitro conditions were exposed to R-DNase I (G-actin probe; a-e) or R-P (F-actin probe) at 3 (pre-confluent; a,f), 6 (confluent; b,g), 10 (4 days post confluent; c,h), 20 (14 days post-confluent; d,i) and 32 (26 days post-confluent; e,j) days of culture. G-actin distribution (a-e) was diffuse with the appearance of enhanced fluorescence near the nucleus (n). Areas of punctate fluorescence (arrows) were also noted, which demonstrated an enhancement of fluorescent intensity by day 20 (d). This continued to increase such that by day 32 (e) most cells possessed a region of intense punctate fluorescence. The F-actin distribution (f) in some pre-confluent cells (y) was diffuse with few if any F-actin stress fibers, while in others (z) such fibers (arrows) were numerous. At confluency (g), cultures demonstrated intense fluorescence with numerous F-actin stress fibers (arrows) that were circumferential or traversed the cells. By day 10 (h), fluorescent intensity was diminished. F-actin stress fibers were fine, not as numerous and some cells (x) appeared to lack fibers that traversed the cell. At 20 days of culture (i), fluorescent intensity was further diminished. F-actin was mainly concentrated in the cell periphery (arrows) with some cells (x) showing little or no fluorescence in the cell interior. No further changes in the F-actin arrangement were noted after 32 days of culture (j). Bar=10μ

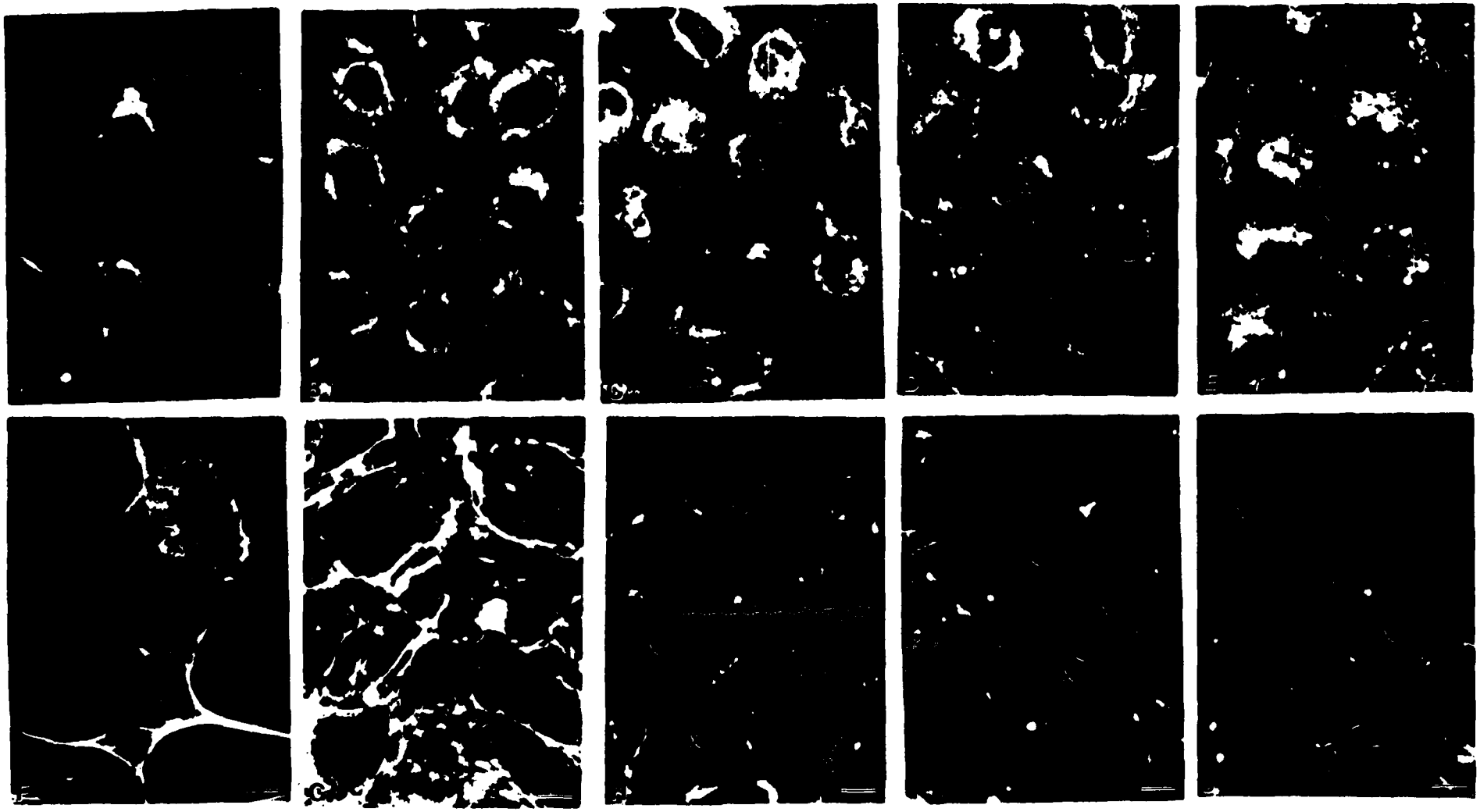

Fig. 3. Post confluent BAEC (day 9) were treated (b,c) with cytochalasin-D (15 min; 10^{-4} M) and then exposed to R-Dnase (G-actin probe; a,b) or R-P (F-actin probe; c,d). The G-actin pool was not affect by cytochalasin-D for both nontreated (a) and treated (b) cells demonstrated a diffuse G-actin distribution, with an area of enhanced fluorescence surrounding the cell nucleus (n). Prior to cytochalasin-D treatment (c), F-actin was distributed in numerous stress fibers that were located in the cell periphery or found to traverse the cell. In addition, some areas of punctate fluorescence (arrows) were noted. After treatment (d), stress fibers were disrupted and many areas of intense diffuse (x) and punctate (arrows) fluorescence were seen. Bar=10μ